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CLEAN VERSION OF PENDING CLAIMS

METHOD FOR INCREASING LUMINESCENCE ASSAY SENSITIVITY
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Serial No.: 09/590,884

1. A method for increasing the sensitivity of a bio-luminescent assay comprising carrying out the assay in the presence of an organic compound that reduces luminescence that is not dependent on the presence of an analyte by at least about 10 fold, and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold.

2. A method for increasing the sensitivity of a luminescent assay comprising carrying out the assay in the presence of an organic compound that reduces luminescence generated by luminogenic molecules not bound to an enzyme by at least about 10 fold, and that reduces the luminescence generated by luminogenic molecules bound to an enzyme by less than about 7 fold.

3. A method for increasing the sensitivity of a bio-luminescent assay comprising carrying out the assay in the presence of an organic compound that reduces autoluminescence by at least about 10 fold, and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold.

4. The method of any one of claims 1-3 wherein the luminescent assay employs a luciferase, aequorin, or obelin enzyme.

5. The method of any one of claims 1-3 wherein the luminescent assay employs firefly luciferase.

6. The method of any one of claims 1-3 wherein the luminescent assay employs *Renilla* luciferase.

7. The method of any one of claims 1-3 wherein the luminescent assay employs *Cypridina* luciferase

8. The method of any one of claims 1-3 wherein the organic compound is present in a concentration of at least 0.1 μM.

9. The method of any one of claims 1-3 wherein the organic compound is present in a concentration of at least 0.1 mM.

10. The method of any one of claims 1-3 wherein the organic compound is present in a concentration of from about 0.1μM to about 500 mM.

11. The method of any one of claims 1-3 wherein the organic compound is present in a concentration of from about 100μM to about 100 mM.

12. The method of any one of claims 1-3 wherein the organic compound is present in a concentration of from about 10 mM to about 100 mM.

13. The method of any one of claims 1-3 wherein the assay is performed in the presence of whole cells.

14. The method of any one of claims 1-3 wherein the assay is carried out in a solvent comprising at least about 10% water by weight.

15. The method of any one of claims 1-3 wherein the assay is carried out in a solvent comprising at least about 25% water by weight.

16. The method of claim 1 wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 5 fold.

17. The method of claim 2 wherein the luminescence generated by luminogenic molecules bound to an enzyme is reduced by less than about 5 fold.

18. The method of claim 3 wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 5 fold.

19. The method of claim 1 wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 2 fold, remains the same, or is increased.

20. The method of claim 2 wherein the luminescence generated by luminogenic molecules bound to an enzyme is reduced by less than about 2 fold, remains the same, or is increased.

21. The method of claim 3 wherein the luminescence that is dependent on the presence of an analyte is reduced by less than about 2 fold, remains the same, or is increased.

22. An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing luminescence that is not dependent on the presence of an analyte by at least about 10 fold, and for reducing luminescence that is dependent on the presence of an analyte by less than about 7 fold.

23. An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing luminescence generated by luminogenic molecules not bound to an enzyme by at least about 10 fold, and for reducing luminescence generated by luminogenic molecules bound to an enzyme by

less than about 7 fold.

24. An assay kit comprising packaging material containing 1) a luminogenic substrate of a luminescent enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing autoluminescence by at least about 10 fold, and for reducing luminescence that is dependent on the presence of an analyte by less than about 7 fold.

25. The kit of any one of claims 22-24 wherein the enzyme substrate and the compound are each contained in a separate container

26. The kit of any one of claims 22-24 wherein the enzyme substrate and the compound are contained in a single container.

27. The kit of any one of claims 22-24 further comprising a buffer solution suitable for use in a luminescent assay.

28. The kit of claim 27 wherein the enzyme substrate and the buffer solution are contained in a single container.

29. The kit of claim 27 wherein the compound and the buffer solution are contained in a single container.

30. The kit of any one of claims 22-24 further comprising a substrate for a second luminescent enzyme.

31. The kit of any one of claims 22-24 further comprising a quenching agent for a luminescent enzyme reaction.

32. The kit of any one of claims 22-24 wherein the substrate is a substrate for firefly luciferase or *Renilla* luciferase.

33. The kit of any one of claims 22-24 further comprising ATP.

34. The kit of any one of claims 22-24 that comprises both a luminogenic substrate of a luminescent enzyme, and a luminogenic enzyme.

35. A method for increasing the sensitivity of a bio-luminescent assay comprising carrying out the assay in the presence of an organic compound that reduces the luminescence that does not result from a bio-luminescent reaction by at least about 10 fold, and that reduces luminescence that does result from a bio-luminescent reaction by less than about 7 fold.

36. The method of claim 1 wherein the luminescence that is dependent on the presence of an analyte is maintained or increases.

37. The method of claim 2 wherein the luminescence generated by luminogenic molecules bound to an enzyme is maintained or increases.

38. The method of claim 3 wherein the luminescence that is dependent on the presence of an analyte is maintained or increases.

39. The method of claim 35 wherein the luminescence that results from a bio-luminescent reaction is maintained or increases.

40. The method of claim 1 wherein the luminescence that is not dependent on the presence of an

analyte is chemi-luminescence that does not result from a bio-luminescent reaction.

41. The method of claim 1 wherein the luminescence that is dependent on the presence of an analyte comprises luminescence generated within a living cell.

42. The method of claim 2 wherein the luminescence generated by luminogenic molecules bound to an enzyme comprises luminescence generated within a living cell.

43. The method of claim 3 wherein the luminescence that is dependent on the presence of an analyte comprises luminescence generated within a living cell.

44. The method of claim 35 wherein the luminescence that does not result from a bio-luminescent reaction comprises luminescence generated within a living cell.

45. The method of claim 1 wherein luminescence that is not dependent on the presence of an analyte comprises luminescence generated by a chemical reaction of coelenterazine or a functional analog thereof.

46. The method of claim 2 wherein the luminescence generated by luminogenic molecules not bound to an enzyme comprises luminescence generated by a chemical reaction of coelenterazine or a functional analog thereof.

47. The method of claim 3 wherein the auto luminescence comprises luminescence generated by a chemical reaction of coelenterazine or a functional analog thereof.

48. The method of claim 35 wherein the luminescence that does not result from a bio-luminescent reaction comprises luminescence generated by a chemical reaction of coelenterazine

or a functional analog thereof.

49. An assay kit comprising packaging material containing 1) a luminogenic substrate of an enzyme, or a luminogenic enzyme; and 2) an organic compound for reducing luminescence that does not result from a bio-luminescent reaction by at least about 10 fold, and that reduces luminescence does result from a bio-luminescent reaction by less than about 7 fold.

50. The assay kit of claim 22 wherein the luminescence that is dependent on the presence of an analyte is maintained or increases.

51. The assay kit of claim 23 wherein the luminescence generated by luminogenic molecules bound to an enzyme is maintained or increases.

52. The assay kit of claim 24 wherein the luminescence that is dependent on the presence of an analyte is maintained or increases.

53. The assay kit of claim 49 wherein the luminescence that results from a bio-luminescent reaction is maintained or increases.

54. The assay kit of claim 22 wherein the luminescence that is dependent on the presence of an analyte comprises luminescence generated within a living cell.

55. The assay kit of claim 23 wherein the luminescence generated by luminogenic molecules bound to an enzyme comprises luminescence generated within a living cell.

56. The assay kit of claim 24 wherein the luminescence that is dependent on the presence of an analyte comprises luminescence generated within a living cell.

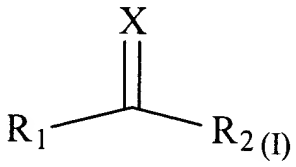
57. The assay kit of claim 49 wherein the luminescence that does not result from a bio-luminescent reaction comprises luminescence generated within a living cell.

58. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound comprises a sulfur atom or a selenium atom.

59. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound contains a carbon-sulfur double bond (C=S).

60. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound contains a carbon-selenium double bond (C=Se).

61. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound is a compound of formula (I):



(I)

wherein X is S or Se; R_1 and R_2 are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_1-C_{20})alkoxy, (C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, heteroaryl, or NR_aR_b; or R_1 and R_2 together with the carbon to which they are attached form a 5, 6, 7, or 8 membered saturated or unsaturated ring comprising carbon and optionally comprising 1, 2, or 3 heteroatoms selected from oxy (-O-), thio (-S-), or nitrogen (-NR_c)-, wherein said ring is optionally substituted with 1, 2, or 3 halo, hydroxy, oxo, thioxo, carboxy, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_1-C_{20})alkoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, (C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or

heteroaryl; and R_a, R_b and R_c are each independently hydrogen, $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl,$(C_2$-$C_{20})$alkenyl, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, $(C_2$-$C_{20})$alkynyl, aryl, heteroaryl; wherein any $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl, $(C_1$-$C_{20})$alkoxy, $(C_2$-$C_{20})$alkenyl $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, or $(C_2$-$C_{20})$alkynyl of R_1 , R_2 , R_a, R_b, and R_c is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more halo, hydroxy, mercapto, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkanoyloxy, sulfo or $(C_1$-$C_{20})$alkoxycarbonyl; or a salt thereof.

62. The method of any one of claims **1, 2, 3, and 35** wherein the organic compound is a compound of formula R_3SH wherein R_3 is $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl,$(C_2$-$C_{20})$alkenyl, $(C_2$-$C_{20})$alkynyl, aryl, or heteroaryl; wherein any $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl, $(C_2$-$C_{20})$alkenyl, or $(C_2$-$C_{20})$alkynyl of R_3 is optionally substituted with one or more halo, hydroxy, mercapto oxo, thioxo, carboxy, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, aryl, heteroaryl, or NR_dR_e; wherein R_d and R_e are each independently hydrogen, $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl,$(C_2$-$C_{20})$alkenyl, $(C_2$-$C_{20})$alkynyl, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkanoyloxy, sulfo or $(C_1$-$C_{20})$alkoxycarbonyl; or a salt thereof.

63. The method of any one of claims **1, 2, 3, and 35** wherein the organic compound is a compound of formula R_4NCS wherein: R_4 is $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl,$(C_2$-$C_{20})$alkenyl, $(C_2$-$C_{20})$alkynyl, aryl, or heteroaryl; wherein any $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl, $(C_2$-$C_{20})$alkenyl, or $(C_2$-$C_{20})$alkynyl of R_4 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, $(C_1$-$C_{20})$alkanoyl, $(C_1$-$C_{20})$alkoxycarbonyl, aryl, heteroaryl, or NR_fR_g; wherein R_f and R_g are each independently hydrogen, $(C_1$-$C_{20})$alkyl, $(C_3$-$C_8)$cycloalkyl,$(C_2$-

C_{20})alkenyl, (C_2-C_{20})alkynyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy, sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

64. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound is a compound of formula R_5-X-R_6 wherein:

X is -S- or -Se-;

R_5 is (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl; and R_6 is hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl;

or R_5 and R_6 together with X form a heteroaryl;

wherein any (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_2-C_{20})alkenyl, or (C_2-C_{20})alkynyl of R_5 or R_6 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, heteroaryl, or NR_kR_m;

wherein R_k and R_m are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, or heteroaryl; and

wherein any aryl or heteroaryl is optionally substituted with one or more halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy, sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

65. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound is a compound of any one of formulae 1-11 of Figure 4.

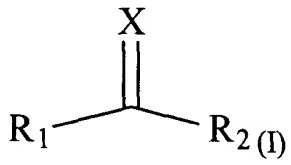
66. The method of any one of claims 1, 2, 3, and 35 wherein the organic compound is a compound of formulae 1, 2, 4, or 6 as shown in Figure 4.

67. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound comprises a sulfur atom or a selenium atom.

68. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound contains a carbon-sulfur double bond (C=S).

69. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound contains a carbon-selenium double bond (C=Se).

70. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound is a compound of
formula (I):



(I)

wherein X is S or Se; R_1 and R_2 are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_1-C_{20})alkoxy, (C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, heteroaryl, or NR_aR_b; or R_1 and R_2 together with the carbon to which they are attached form a 5, 6, 7, or 8 membered saturated or unsaturated ring comprising carbon and optionally comprising 1, 2, or 3 heteroatoms selected from oxy (-O-), thio (-S-), or nitrogen (-NR_c)-, wherein said ring is optionally substituted with 1, 2, or 3 halo, hydroxy, oxo, thioxo, carboxy, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_1-C_{20})alkoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, (C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl; and R_a, R_b and R_c are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, (C_2-C_{20})alkynyl, aryl, heteroaryl; wherein any (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_1-C_{20})alkoxy, (C_2-C_{20})alkenyl (C_1-

C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, or (C_2-C_{20})alkynyl of R_1 , R_2 , R_a, R_b, and R_c is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more halo, hydroxy, mercapto, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy, sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

71. **The kit of any one of claims 22, 23, 24, and 49** wherein the organic compound is a compound of formula R_3SH wherein R_3 is (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl; wherein any (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_2-C_{20})alkenyl, or (C_2-C_{20})alkynyl of R_3 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, heteroaryl, or NR_dR_e; wherein R_d and R_e are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy, sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

72. **The kit of any one of claims 22, 23, 24, and 49** wherein the organic compound is a compound of formula R_4NCS wherein: R_4 is (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl; wherein any (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_2-C_{20})alkenyl, or (C_2-C_{20})alkynyl of R_3 is optionally substituted with one or more halo, hydroxy, mercapto oxo, thioxo, carboxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, heteroaryl, or NR_fR_g; wherein R_f and R_g are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl aryl, or heteroaryl; and wherein any aryl or heteroaryl is optionally substituted with one or more (1, 2, 3, or 4) halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy,

sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

73. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound is a compound of formula R_5-X-R_6 wherein:

X is -S- or -Se-;

R_5 is (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl; and R_6 is hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, aryl, or heteroaryl;

or R_5 and R_6 together with X form a heteroaryl;

wherein any (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl, (C_2-C_{20})alkenyl, or (C_2-C_{20})alkynyl of R_5 or R_6 is optionally substituted with one or more halo, hydroxy, mercapto, oxo, thioxo, carboxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, heteroaryl, or NR_kR_m;

wherein R_k and R_m are each independently hydrogen, (C_1-C_{20})alkyl, (C_3-C_8)cycloalkyl,(C_2-C_{20})alkenyl, (C_2-C_{20})alkynyl, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkoxycarbonyl, aryl, or heteroaryl; and

wherein any aryl or heteroaryl is optionally substituted with one or more halo, mercapto, hydroxy, oxo, carboxy, cyano, nitro, trifluoromethyl, trifluoromethoxy, (C_1-C_{20})alkanoyl, (C_1-C_{20})alkanoyloxy, sulfo or (C_1-C_{20})alkoxycarbonyl; or a salt thereof.

74. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound is a compound of any one of formulae 1-11 as shown in Figure 4.

75. The kit of any one of claims 22, 23, 24, and 49 wherein the organic compound is a compound of formula 1, 2, 4, or 6 as shown in Figure 4.

76.(NEW) A method for increasing the sensitivity of an assay comprising carrying out a bioluminescent reaction in the presence of an organic compound that reduces luminescence that

is not dependent on the presence of an analyte by at least about 10 fold, and that reduces luminescence that is dependent on the presence of an analyte by less than about 7 fold.

77.(NEW) A method for increasing the sensitivity of an assay comprising carrying out the assay in the presence of an organic compound that reduces luminescence generated by luminogenic molecules not bound to a bioluminescent enzyme by at least about 10 fold, and that reduces the luminescence generated by luminogenic molecules bound to a bioluminescent enzyme by less than about 7 fold.

78.(NEW) A method for increasing the sensitivity of an assay comprising carrying out a bioluminescent reaction in the presence of an organic compound that reduces autoluminescence by at least about 10 fold, and that reduces luminescence that is dependent on the presence of a bioluminescent enzyme by less than about 7 fold.